software packages such as the Wisconsin Package™ (Genetic Computer Group, Madison, Wisconsin) include programs such as FRAMES and CodonPreference that help to identify protein coding sequences in a query nucleotide sequence. FRAMES displays open reading frames for the six DNA translation frames, allowing one to quickly assess the presence or absence of stretches of open-reading frames that are likely to be protein encoding regions. CodonPreference is a more sophisticated program that identifies and displays possible protein coding regions based on similarity of the codon usage in the sequence to a codon frequency table (Gribskov et al., 1984).

EXAMPLE 1: Differential Gene Expression Analysis in Pine Tree Embryogenesis

[0134] cDNA libraries were prepared from staged pine tree embryos, as described above. The differential display technique was used to identify 327 novel cDNAs that were preferentially-expressed during early, middle, or late stages of pine tree embryogenesis, as set forth below. Clone nomenclature is divided into subsets based on tissue type; a clone is designated LPS to indicate somatic origins and LPZ for zygotic origins.

Plant Materials

[0135] Somatic embryos were collected at different stages of development. Cultures of somatic embryos of were initiated from Loblolly pine immature zygotic embryos as described by Becwar et al., *Forestry Science* 44:287-301 (1994) (incorporated by reference) or with minor modifications in media mineral composition. Somatic embryos were grown in cell suspension culture medium 16 (Pullman and Webb, Tappi R&D Division 1994 Biological Sciences Symposium) and a maturation medium similar to that of a standard maturation media. Resulting somatic embryos

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were selected and classified as stages 1-9 according to morphological development following the teachings of Pullman and Webb, Tappi R&D Division 1994 Biological Sciences Symposium pp.31-34. Somatic embryos were sorted into tubes containing the same stages and stored at -70 °C.

RNA Isolation

[0136] Total RNA was isolated from all stages of somatic embryos of loblolly pine and grouped into early, middle, and late phases of development. The early phase is represented by a liquid suspension culture containing embryos of stages 1 through stage 3. Middle phase contains embryos of stages 4 through stage 6, while stages 7 through 9 formed the late phase. 60-100 mg aliquots of staged frozen embryos were ground in 1.0 ml of TRI Reagent® Isolation Reagent (Molecular Research Center, Inc.), a commercial product that includes phenol and guideline thiocyanate in a monophase solution and extracted according to the manufacturer's instructions.

Reverse Transcription of mRNA (RT-PCR)

[0137] The total RNA was used as a template to synthesize single stranded DNA mediated by MMLV reverse transcriptase (100 U/µl). The method involves the reverse transcription by PCR of the rnRNA with an oligo-dT primer (H-T_{11}G: 5' B AAGCTTTTTTTTTTTG 3') anchored to the beginning of the poly(A) tail, followed by a PCR reaction in the presence of a second short (13-mer) primer which is arbitrary in sequence [AP_1 (5' B AAGCTTGATTGCC - 3') or AP_2 (5' B AAGCTTCGACTGT - 3')]. Reverse transcription and Differential Display were conducted using the GenHunter RNAimage Kit I.

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[0138] A 19 µl reverse transcription reaction (10 µl sterile water, 2.0 µl 5x RT buffer, 1.6 µl dNTP (250 µM), 2.0 µl anchored primer (2.0 µM), 2.0 µl RNA template at 100 ng/µl) was prepared for each embryo phase sample. The reaction mixture was heated to 65°C for 5 minutes in a thermocycler, cooled to 37°C and paused after 10 minutes while 1.0 µl MMLV was added. The program was allowed to resume at 37°C for 50 minutes. The reaction was then heated to 75°C for 5 minutes, cooled to 4°C and stored at -20°C.

Incorporation of Radiolabeled Nucleotides by PCR

[0139] Differential Display PCR was performed in a 20 µl reaction containing 2 µl of the reverse-transcribed cDNA template; 10 µl sterile water 2.0 µl 10x PCR buffer, 1.6 µl dNTP (25 µM), 2.0 µl anchored primer H-T11 G, (2.0 µM), 2.0 µl 13 mer arbitrary primer (AP_1 or AP_2 (2.0 µM), 0.2 µl Taq DNA polymerase, and 0.2 µl α^{32}P-dATP (2000 Ci/mmole). The cDNA was amplified by PCR: 94°C for 3 minutes, 40 cycles of 94°C for 30 seconds, 40°C for 2 minutes, and 72°C for 30 seconds, followed by 72°C for 5 minutes. The reaction was cooled to 4°C and stored at -20°C.

Differential Display

[0140] The PCR products were separated on a Stratagene (La Jolla, California) pre-cast 6% polyacrylamide sequencing gel at 30 watts constant power for approximately 2.5 to 3 hours. 3.5 µl of sample was mixed with 2.0 µl of loading dye and incubated at 80°C for 2 minutes immediately before loading onto the gel. The gel was rinsed in water and dried. Dilute ^{33}P-dATP with loading dye was spotted at the corners

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